

Role of sooty mold fungi in accumulation of fine-particle-associated PAHs and metals on deciduous leaves

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Abstract

The focus of this research was on elucidation of the role of deciduous tree ecosystems in accumulation of fine-particle-associated polycyclic aromatic hydrocarbons (PAHs) and heavy metals on leaves of deciduous trees. The studied species were *Tilia x euchlora* (frequently infested by sooty mold fungi) and *Pyrus calleryana* (unaffected by sooty mold fungi). The selected species have similar leaf morphology and were exposed to identical environmental conditions. Intra-species comparison showed that moldy linden leaves accumulate significantly higher amounts of PAHs and metals than unaffected linden leaves. Inter-species comparison revealed that in the absence of sooty mold fungi, physico-chemical properties of epicuticular waxes, rather than the amounts of waxes, might play an important role in accumulation of particulate matter on leaves.

The accumulation and/or degradation of a number of high-molecular-weight (HMW) PAHs on leaves was temperature dependent. The results show that the presence of sooty mold fungi on deciduous leaves alters either the accumulation modes and/or degradation pathways of PAHs on deciduous leaves.

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1. Introduction

It is commonly accepted that vegetation plays an important role in removal of atmospheric pollutants (Beckett et al., 1998; Paterson et al., 1990). The interpretations of the accumulation of air pollutants on vegetative surfaces are rather dissimilar. Wania and McLachlan (2001) estimated the influence of forests on the overall fate of semi-volatile organic compounds (SVOCs) using a Multimedia Fate Model. The investigators concluded that a high degradation loss on the plant surface had the effect of preventing the saturation of the

small plant storage reservoir and could cause very significant reductions in atmospheric concentration of SVOCs. McLachlan (1999) emphasized the role of the phase in which a compound exists in the atmosphere and developed a framework for the interpretation of measurements of SVOCs in plants. The framework took into account three processes through which plants could accumulate SVOCs: equilibrium partitioning between the plant surface and the gas phase, kinetically limited gaseous deposition, and wet or dry particle-bound deposition. The author suggested that for a volatile compound the equilibrium between gaseous phase and vegetative surface might be achieved in a much shorter time (a day) than for a less volatile compound (a month). Little accumulation was predicted for compounds that fell into a kinetically limited gaseous deposition mode.

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It has also been shown that different species of trees can accumulate various amounts of atmospheric air pollutants. The explanations of inter-species differences in entrapment of the pollutants on plant surfaces vary. Smith (1981) attributed the observed differences to morphological differences of leaves. Research work of Bohme et al. (1999), Komp and McLachlan (1997), Harner et al. (1999), and Jouraeva et al. (2002) points to the fact that the lipophilicity of a plant storage compartment had different physicochemical properties than octanol, commonly used to approximate properties of epicuticular waxes.

Since more than 80% of the forest in the northeast US is deciduous (Alerich and Drake, 1995), the focus of this research was on deciduous tree leaves. Jouraeva et al. (2002) concluded that the elements of the deciduous tree ecosystem, such as aphids and sooty mold fungi, might be contributing to the differences in the amounts of air pollutants on leaves.

Sooty molds are dark-pigmented fungi which are non-parasitic, saprophytic, and superficial on plants. Some of the common genera of fungi found in sooty mold complexes are *Cladosporium*, *Aureobasidium*, *Fumago*, *Antennariella*, *Limacinula*, *Scorias*, and *Capnodium* (Encyclopedia Britannica, 2004; Sinclair et al., 1987; Hughes, 1976; Reynolds, 1976). These fungi tend to assume various shapes and frequently grow on honeydew (a mixture of minerals, sugars, aminoacids, proteins, and other organic compounds (Auclair, 1963) excreted by sucking insects—aphids or scales. Sooty molds are more common under warm conditions and prefer an acidic environment for their growth. A number of studies show that increased concentrations of sulfur and nitrogen oxides in the air, higher temperatures, and increased drought stress enhance the reproduction of aphids, and consequently, occurrence of sooty molds (Dohmen, 1988; Bolsinger and Fluckiger, 1987; Bewley, 1980). Some sooty mold fungal species are specific to a particular plant or insects, and others may colonize many types of surfaces.

Polycyclic aromatic hydrocarbon (PAHs), environmental pollutants that are associated with fine particulate matter, were chosen to study the role of sooty mold fungi in accumulation/decomposition of SVOCS on leaves. The burden of PAHs on leaves depends on environmental conditions—temperature, humidity, UV radiation, and wind. The chemical composition of particles and their surface properties, determined by the type of fuel and the conditions of fuel combustion, also play an important role in decomposition of PAHs adsorbed to the particles (Odum et al., 1994; Fan et al., 1996; Kamens et al., 1994; McDow et al., 1994, 1996). Kotzias and Brussol (1999) and Gerasimov (2004) reported that nitration of PAHs was favored when the compounds were adsorbed onto particles rather than in the gas phase. Menichini et al. (1999) simultaneously measured particle-bound carcinogenic PAHs, CO, and NO_x at a street site. CO, NO, and NO_x were parameters strongly correlated with PAH concentrations ($R = 0.92$). Drastic reduction in the correlation was

observed for summer months only. The authors suggested that this might be the combined effect of high temperature and sunlight intensity.

Kamens et al. (1986) showed that the overall initial rate of PAH decay on soot particles could be approximated by a first order rate expression. The authors suggested that temperature can strongly influence photo-induced degradation of PAHs. This suggestion was supported by research results of Park et al. (2002). The authors concluded that photo-oxidation of PAHs was not an important factor in variation of PAH air concentrations in summer and that temperature and relative humidity had a greater effect on the decay of high-molecular-weight (HMW) PAHs than on the degradation of medium-molecular-weight (MMW) PAHs.

Few of the studies describing the attenuation of air pollutants by vegetation took into account the contribution of the micro-scale ecosystems surrounding vegetation. Sooty mold fungi's ability to trap atmospheric particles has not been widely investigated. Sooty mold fungi are not thought of as having any beneficial environmental effects and are ruthlessly exterminated for the sake of esthetics and to protect the health of the growing number of people with allergies. Sooty mold fungi can be indirectly controlled by reducing populations of insects that excrete honeydew or by using fungicides (Decoin, 2000; Ravesloot et al., 1999; Zuparko, 1995). Excessive use of pesticides, however, might be the reason for the increased number of incidences of asthma and allergies, especially among children (Richter-Reichhelm et al., 2002; Petrie et al., 2003; Phillips, 2000). This research shows that sooty mold fungi-infested trees can remove higher amounts of fine particles containing toxic and often carcinogenic compounds than unaffected species. The role of temperature in degradation of particulate and/or gaseous PAHs trapped on leaves was also examined in this research as one of the ways to observe the role of sooty mold fungi in accumulation and/or degradation of PAHs on deciduous leaves.

2. Materials and methods

2.1. Samples

Tilia x euchlora (linden) and *Pyrus calleryana* (pear) tree leaves were collected during the growing season of 2000 (at the end of each month from May through October). *Tilia x euchlora* is a hybrid developed in the 19th century. Its origin is undecided, but it is believed to be *Tilia cordata* and *Tilia clasystyia* (Little, 1980).

Eight trees (four trees of each species) at each of two study sites were situated next to each other and were subjected to similar environmental conditions, which allowed elimination of the effect of weather on accumulation of toxins. Since the two collection sites (University Place, Syracuse University campus and the corner of East Genesee St. and Irving Ave., Syracuse, NY) were located on the city bus routes, the trees were exposed to automobile and diesel exhausts. Leaves were collected at the same height; about 2m above ground level, and the ambient air temperature was recorded during each sampling. To assure sampling of leaves that had comparable exposures to air pollutants, the leaves were

taken from the middle portion of the branch (only leaves that emerged in early spring). To help account for variability, leaves were collected from all sides of the study trees. The samples were stored at -20°C in solvent-rinsed glass jars until the analysis.

To test the temperature dependence in accumulation of toxins on leaves, samples collected throughout the growing season of 2000 were used. To test the importance of sooty mold fungi in the accumulation, only samples collected at the end of June and in the middle of July (prior to complete infestation by molds) were used. For these latter samples, the linden leaves were separated into two groups: moldy linden (ML) leaves with obvious black sooty molds covering their surface and clean linden (CL) leaves with no apparent mold growth based on a judgment of an analyst. Pear (P) tree leaves were not infested by sooty mold fungi or aphids (precursors of molds).

2.2. Materials

Sixteen PAHs, EPA priority pollutants (PAH standards, 98% purity, Ultra Scientific), were used in this experiment (abbreviations are given in parentheses): naphthalene (Nap), acenaphthylene (Ay), acenaphthene (Ae), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flr), pyrene (Pyr), benz(a)anthracene (B(a)a), chrysene (Cry), benzo(b)-fluoranthene (B(b)f), benzo(k)fluoranthene (B(k)f), benzo(a)pyrene (B(a)p), indeno(1,2,3-cd)pyrene (Ind), dibenz(a,h)anthracene (Di), and benzo(ghi)perylene (B(g,h,i)p). The internal standard (IS), benzo(b)fluorene, was 95% pure (Ultra Scientific). Metals studied were lead, copper, and iron. Chemicals were purchased from Fisher or VWR Scientific (USA) in the highest purity grade available.

2.3. Analyses

2.3.1. PAHs

Ten micrograms of internal standard were added to samples prior to any analytical procedure. Two replicates of composite samples (50.0 g of fresh leaves each, for each sampling date) were soaked with 200 mL of 2 M KOH in methanoic solution (methanol to water, 9:1) for 3 h. The extract was diluted with 200 mL of water. This mixture was subjected to partition extraction with 100 mL of hexane, followed by two additional extractions, 50-mL each, with hexane. Hexane extracts were combined and washed twice with 200 mL of water, dried with anhydrous sodium sulfate overnight, and evaporated to 1 mL with a Kuderna-Danish evaporator. The concentrated extract was subjected to separation of PAHs from constituents of epicuticular waxes using a Silica gel column. Hexane and 25% dichloromethane in hexane were used as eluents. The fraction containing PAHs was evaporated to a volume of 0.5 mL by bubbling nitrogen gas through the sample and analyzed by gas chromatography/mass spectroscopy (GC-MS). Quality control samples were run after every 6th sample. Detailed methods and procedures utilized in the experiments are described in Jouraeva et al. (2002).

2.3.2. Metals

Ten grams of fresh leaves (two replicates for each sampling date and species) were shaken in 75.0 mL of 5% HNO_3 acid solution on a reciprocal shaker for 90 min. The samples were then filtered. Blanks and standards were prepared using 5% HNO_3 and quality control samples were run after every seventh analysis. Samples were analyzed for Cu, Fe, and Pb, using an ICP-AES (Inductively coupled plasma-atomic emission spectrometer) with background correction. Detailed methods and procedures utilized in the experiments are described in Jouraeva et al. (2002).

2.4. Leaf areas and dry weights

For each sampling date the leaf areas of 25.0 g of fresh leaves were measured using a MetaMorph Image Analysis program (Universal Imaging Corporation). Dry weights of the leaves were determined by drying the leaves at 105°C for 24 h with subsequent cooling in a desiccator

and weighing. These measurements were used to report the amounts of contaminants per unit of leaf surface area (i.e., ng of PAH/dm² and μg of metal/dm²). These units were used to avoid the effects of decrease of dry weight of leaves in late summer–early fall, growth dilution, and misinterpretation of data due to various amounts of biomass per leaf area of study trees. It is worth noting that pear tree leaves had a higher amount of epicuticular waxes per unit of leaf surface area than linden leaves and a significantly lower surface-area to dry-weight monthly ratios (Jouraeva et al., 2002).

2.5. Scanning electron microscope (SEM) images

A SEM, JEOL JSM 5800LV, with energy dispersive X-ray analyzer and a backscattered detector was used to take secondary and backscattered electron (BSE) images.

2.6. Statistical analyses

A paired two sample *T*-test for means (paired by the collection dates for the same site) was used to compare the mean concentrations of PAHs and metals on leaves collected at the end of June and middle of July of 2000 at 95% confidence level. Each mean was the average of three samples. This allowed intra- and inter-species assessments.

Linear regression analysis at 90% confidence level was used to determine whether or not the accumulation of PAHs on leaves of the two species was temperature dependent. Mean PAH concentrations of samples collected throughout the entire growing season of 2000 were plotted vs. corresponding sampling time temperatures (*T*, $^{\circ}\text{C}$). Other temperature data utilized in the statistical analysis were obtained at <http://www.nws.noaa.gov> and included: the lowest *T* of the sampling date, the average *T* of the sampling date, and the temperatures averaged over the periods of 2, 3, 4, and 5 days prior to sampling. *P*-value, mentioned throughout the paper, is the probability of wrongly rejecting the null hypothesis (*H*₀) if it is in fact true.

3. Results and discussion

3.1. The importance of sooty mold fungi in accumulation of PAHs and metals on leaves

At the beginning of the growing season, linden (but not pear) leaves were infested by the linden aphid, *Eucallipterus tiliæ*, and had numerous honeydew deposits. Honeydews serve as fertilizers for sooty mold fungi that appear later in the growing season. A BSE image of the linden leaf surface sampled at the beginning of the growing season (Fig. 1) shows honeydew deposits (white arrows) and particles loaded with heavy metals (bright white spots). As can be seen from the picture, fine particles are not deposited to the honeydew surface. Some bright spots at the edges of honeydew spots correspond to particles that were trapped by the waxy leaf surface prior to deposition of honeydew. It appears that honeydew deposits mask the waxy leaf surface, preventing it from trapping particles. Perhaps, this masking is responsible for a reduced rate of accumulation of lead on linden leaves compared to that of on pear leaves between June and July sampling dates (Fig. 2, white solid area). At the same time the mold growth on linden leaves must have boosted the abundance of fine particles on leaves, as a result the concentration of lead on linden leaves increased from July to August. The BSE image (Fig. 3) of a

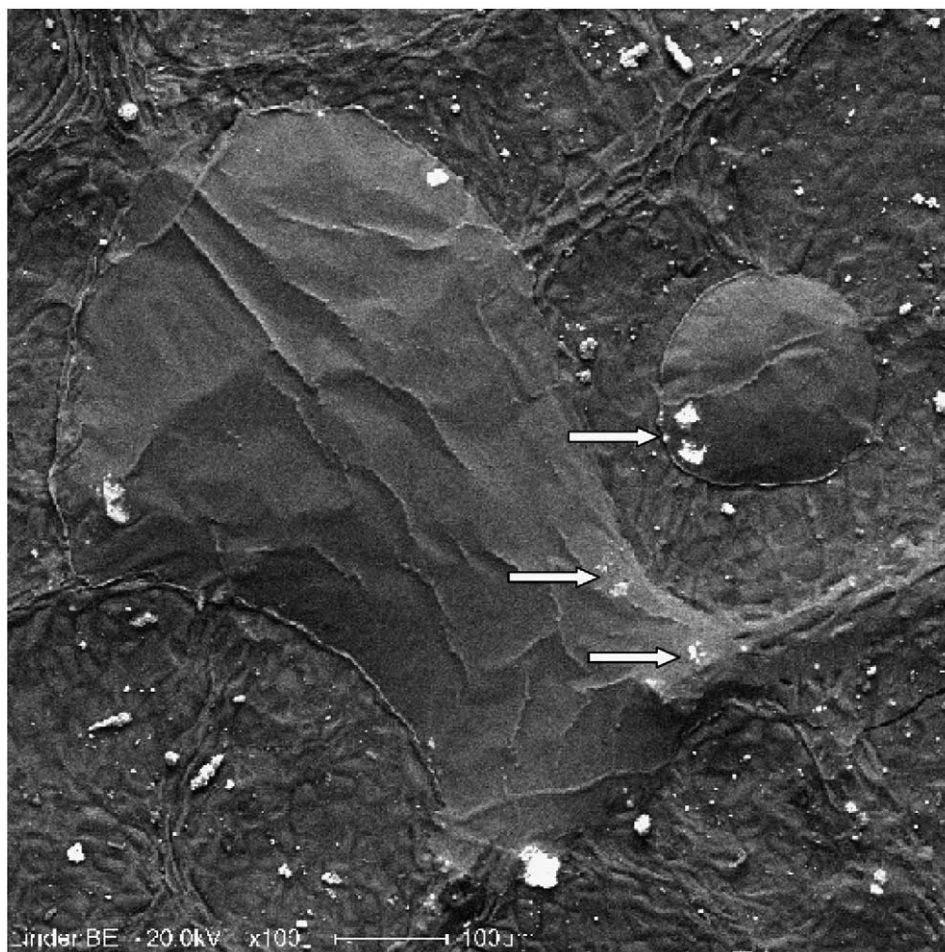


Fig. 1. Backscattered electron image of honeydew deposits on *Tilia x euchlora* leaves. Bright white spots throughout the leaf surface correspond to particles containing heavy metals. The arrows point to the particles covered by honeydew deposits. The scale bar is 100 μm .

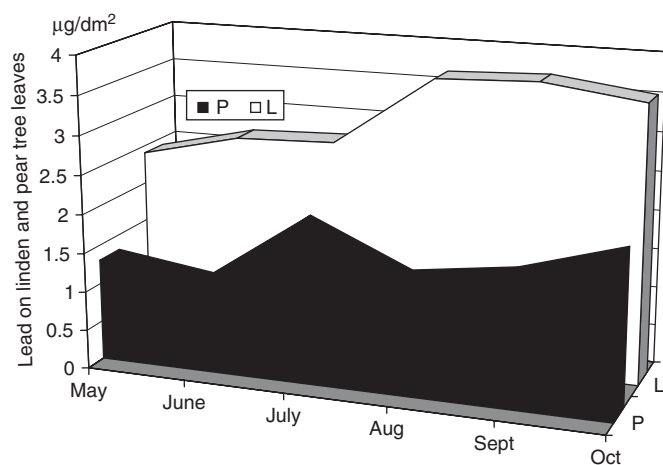


Fig. 2. Lead on leaves of *Tilia x euchlora* (L) and *Pyrus calleryana* (P) over the growing season of 2000.

linden leaf (mid-July) reveals that a large surface area of sooty mold fungi traps numerous fine particles, while a few particles are deposited on bare leaf surfaces indicated by white arrows.

The amount of PAHs on linden leaves did not increase in September–October, most likely because the rate of deposition of fine particles to and erosion from the leaves were similar. Weathering of waxy leaf surface and reduced amounts of honeydew on leaves (washed off by rain and/or consumed by sooty mold fungi) might have slowed down the fungi growth during the last two months of the growing season. To elucidate the importance of sooty mold fungi in accumulation of PAHs, both intra- and inter-species differences were examined using a paired sample *T*-test for means. The results are presented in Table 1. The results indicate that concentrations of 15 out of 16 studied PAHs on ML leaves were significantly higher than on clean (non-moldy) linden (CL) leaves ($\mu[\text{PAH}]_{\text{ML}} - \mu[\text{PAH}]_{\text{CL}} > 0$) at 95% confidence level. Mean concentrations of fluorine on ML and CL were not significantly different.

Inter-species differences showed that CL leaves accumulated significantly higher amounts of HMW PAHs than pear (P) leaves ($\mu[\text{PAH}]_{\text{CL}} - \mu[\text{PAH}]_{\text{P}} > 0$). This difference suggests that the composition of epicuticular waxes plays an important role in removal of atmospheric particle-bound pollutants from the air. Indeed, *Tilia* genus waxes (Gulz et al., 1988; Gulz, 1994) have a very different

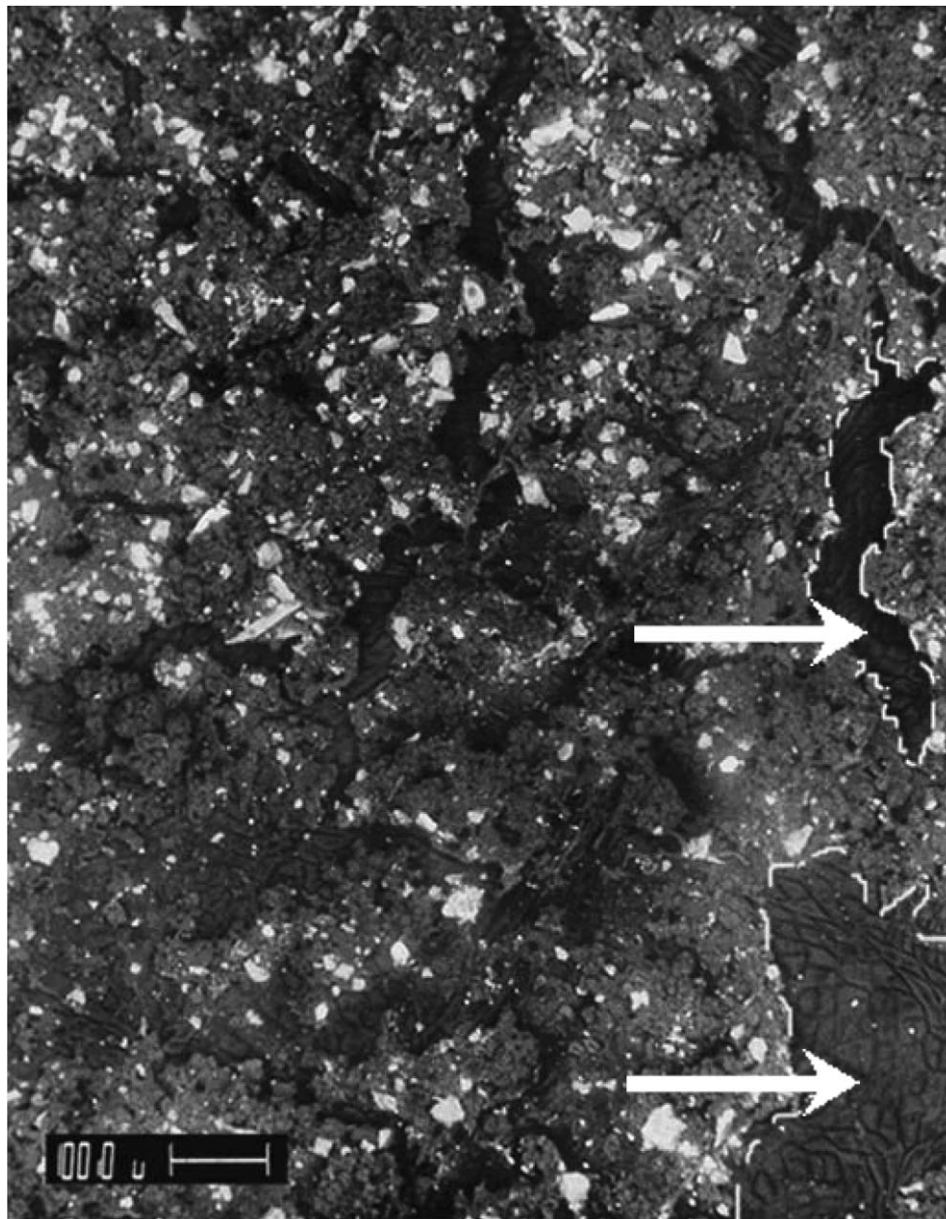


Fig. 3. Backscattered electron image of a moldy *Tilia x euchlora* leaf surface. The dashed areas indicated by the arrows correspond to the leaf surface areas unaffected by sooty mold fungi growth. The bright white spots correspond to metal containing particles. The scale bar is 100 μm .

composition with much greater amounts of triterpenoids than waxes of *Pyrus* genus (Challice et al., 1980). More research is needed to determine the exact make-up of waxes and identify compounds that might be responsible for adhesiveness of linden tree leaf epicuticular waxes.

For volatile low-molecular-weight (LMW) compounds: Nap, Ay, Ace, Flu, and Ant, the concentrations of Ay and Flu on pear leaves were significantly greater than on CL leaves at 95% confidence level (P -values for H_0 were 0.97 and 0.99, correspondingly). This may be due to the fact that equilibrium partitioning (a primary mode of deposition of these chemicals on leaves) is proportional to the amount of waxes on leaves and to the available waxy leaf surface area (McLachlan, 1999). Both of these parameters are higher for P: pear leaves have more wax on their

surface: pear ($0.70 \pm 0.09 \text{ mg/dm}^2$) and linden ($0.51 \pm 0.02 \text{ mg/dm}^2$); P -value = 0.046 (Jouraeva et al., 2002); and linden leaves are either covered by honeydew or sooty molds, which reduces their surface area. Concentrations of all three studied metals were also significantly higher on CL leaves than on P leaves. This confirms the need for further research of physicochemical properties of epicuticular waxes of deciduous tree leaves.

It was also determined that chrysene, a compound that falls into a kinetically-limited partitioning mode of accumulation, did not show a significant inter-species variability at 95% confidence level (P -value = 0.09). This finding is in agreement with Bohme's study (Bohme et al., 1999), which showed that there was no inter-species variability in chrysene concentrations in ten plant species.

Table 1
Results of a paired two sample *T*-test for PAHs (June and July sampling dates)

PAHs	Mean concentrations of PAHs \pm SE (ng/dm ²)			<i>P</i> -values $\alpha = 0.05$	
	ML	CL	P	ML > CL ^a	CL > P ^b
Naphthalene	1.6 \pm 0.2	1.1 \pm 0.1	1.2 \pm 0.2	0.02^c	0.80
Acenaphthylene	0.3 \pm 0.0	0.2 \pm 0.0	0.4 \pm 0.1	0.01	0.97
Acenaphthene	2.0 \pm 0.2	1.2 \pm 0.2	1.9 \pm 0.3	0.01	0.87
Fluorene	3.5 \pm 0.4	2.4 \pm 0.5	4.9 \pm 0.3	0.10	0.99
Phenanthrene	48.8 \pm 4.7	37.8 \pm 2.6	29.8 \pm 2.7	0.02	0.04
Anthracene	2.81 \pm 0.29	1.9 \pm 0.3	2.3 \pm 0.3	0.04	0.95
Fluoranthene	51.0 \pm 4.7	30.7 \pm 4.2	22.0 \pm 1.1	0.05	0.04
Pyrene	32.4 \pm 2.9	19.0 \pm 2.3	14.3 \pm 1.2	0.03	0.03
Benzo(a)anthracene	10.4 \pm 1.1	6.7 \pm 1.1	3.7 \pm 0.2	0.02	0.03
Chrysene	20.2 \pm 1.9	16.0 \pm 1.1	13.6 \pm 0.8	0.01	0.09
Benzo(b)fluoranthene	14.4 \pm 1.6	8.8 \pm 1.4	4.7 \pm 0.3	0.02	0.04
Benzo(k)fluoranthene	14.5 \pm 1.5	9.6 \pm 1.4	6.4 \pm 0.3	0.02	0.05
Benzo(a)pyrene	11.5 \pm 1.2	6.7 \pm 1.1	3.2 \pm 0.1	0.02	0.02
Indeno(1,2,3-cd)pyrene	9.6 \pm 1.1	5.7 \pm 1.2	3.5 \pm 0.2	0.02	0.04
Benzo(g,h,i)perylene	8.9 \pm 1.0	5.7 \pm 0.9	3.1 \pm 0.1	0.02	0.04
Dibenzo(a,h)anthracene	1.8 \pm 0.2	1.1 \pm 0.2	0.6 \pm 0.1	0.03	0.02

Concentrations of PAHs on moldy linden leaves are presented in the column labeled, ML; on clean, non-infested by molds, linden leaves, CL; and on pear tree leaves, P.

^a $\mu[\text{PAH}]_{\text{ML}} - \mu[\text{PAH}]_{\text{CL}} = 0$ vs. $\mu[\text{PAH}]_{\text{ML}} - \mu[\text{PAH}]_{\text{CL}} > 0$.

^b $\mu[\text{PAH}]_{\text{CL}} - \mu[\text{PAH}]_{\text{P}} = 0$ vs. $\mu[\text{PAH}]_{\text{CL}} - \mu[\text{PAH}]_{\text{P}} > 0$.

^c Results in bold indicate significant difference.

However, the presence of sooty mold fungi substantially increased the burdens of chrysene on linden leaves ($\mu[\text{Cry}]_{\text{ML}} - \mu[\text{Cry}]_{\text{P}} > 0$, *P*-value = 0.04).

Studied metals were used as fine particle tracers since most of the metal burden in vegetation is deposited on its surface (Little, 1973; Alshayev et al., 1995; Krivan et al., 1987). According to the US EPA (2004) and Schroeder et al. (1987), lead is predominantly present in fine fraction of particular matter (less than 2.5 μm in aerodynamic diameter, $\text{PM}_{2.5}$); while Fe and Cu can occur in both: $\text{PM}_{2.5}$ and coarse (less than 10 μm in aerodynamic diameter, PM_{10}) fractions. Comparisons of metals on ML vs. CL and on CL vs. P leaves for end-of-June and mid-July samples are presented in Table 2. Concentrations of lead, iron, and copper were significantly higher on ML leaves than on CL leaves. The results confirm that sooty mold fungi are excellent collectors of both fine (lead, $\text{PM}_{2.5}$) and coarse (copper and iron, PM_{10}) atmospheric particles.

3.2. Temperature-induced changes in accumulation of PAHs and metals on leaves

3.2.1. Metals on leaves

Accumulation of metals on leaves of both species was not temperature dependent. In spite of the differences in accumulation patterns of lead on leaves of the two species, the amount of fine-particle-associated lead increased over the growing season on both linden and pear leaves (Fig. 2). In the absence of any chemical partitioning forces for the surface or chemical/photochemical degradation of metals,

the association seems to be simply related to the leaf surface physical properties.

3.2.2. PAHs on *Tilia x euchlora* leaves

Even though PAHs are also associated with fine particles, their seasonal trends were different from that of lead. Fig. 4 depicts the temperature-dependent trend for benzo(a)pyrene over the growing season. Substantial reduction in PAH concentrations was observed during the warmest summer months (June and July). This was more evident for PAHs accumulated on linden than on pear tree leaves and more pronounced for HMW and MMW PAHs.

The reduction of the amounts of PAHs on leaves might be correlated with their reduced concentrations in the air. Such reductions in air concentrations during summer months have recently been observed by several investigators (Eiguren-Fernandez et al., 2004; Mastra et al., 2003; Lodovici et al., 2003; Guo et al., 2003; Cancio et al., 2001; Dimashki et al., 2001). However, these researchers proposed different explanations for their observations.

Eiguren-Fernandez et al. (2004) attributed negative correlation of particle-phase PAHs with mean air temperature in urban sites to surface inversions occurring during winter. Guo et al. (2003) suggested that the lower summer PAH concentrations were likely due to easier dispersion of air pollutants, washout effects, and to a lesser extent, photo-degradation and higher percentage of PAHs in vapor than in the particulate phase. Cancio et al. (2001) also suggested the particle-to-gas conversion process as a possible explanation for the reduced air concentrations of

Table 2
Results of a paired two sample *T*-test for metals (June and July sampling dates)

Metals	Mean concentrations of metals \pm SE ($\mu\text{g}/\text{dm}^2$)			<i>P</i> -values $\alpha = 0.05$	
	ML	CL	P	ML > CL ^a	CL > P ^b
Pb	2.91 \pm 0.07	1.35 \pm 0.02	0.82 \pm 0.01	0.00 ^c	0.01
Cu	2.33 \pm 0.16	1.94 \pm 0.13	1.22 \pm 0.30	0.05	0.05
Fe	26.83 \pm 1.20	17.69 \pm 0.47	10.30 \pm 0.76	0.00	0.01

Concentrations of metals on moldy linden leaves are presented in the column labeled, ML; on clean, non-infested, linden leaves, CL; and on pear tree leaves, P.

^a $\mu[\text{Metals}]_{\text{ML}} - \mu[\text{metals}]_{\text{CL}} = 0$ vs. $\mu[\text{metals}]_{\text{ML}} - \mu[\text{metals}]_{\text{CL}} > 0$.

^b $\mu[\text{Metals}]_{\text{CL}} - \mu[\text{metals}]_{\text{P}} = 0$ vs. $\mu[\text{metals}]_{\text{CL}} - \mu[\text{metals}]_{\text{P}} > 0$.

^cResults in bold indicate significant difference.

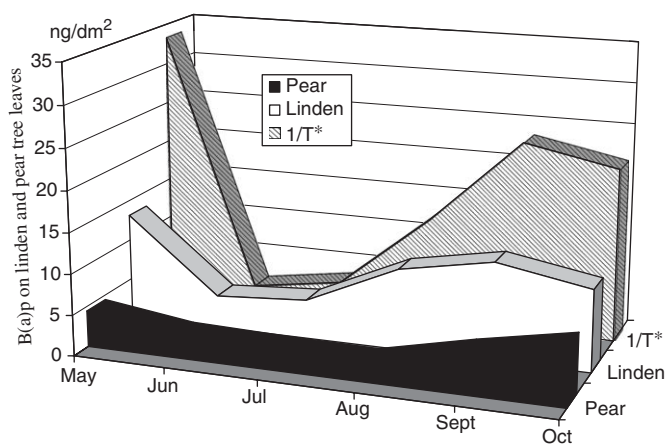


Fig. 4. Benzo(a)pyrene on leaves of *Tilia x euchlora* and *Pyrus calleryana* over the growing season of 2000. **T* ($^{\circ}\text{C}$) is average temperature of the sampling day adjusted to the scale.

PAHs. Dimashki et al. (2001) plotted concentrations of PAHs divided by the corresponding nitrogen monoxide (NO) air concentrations against temperatures and detected no significant relationship for any PAH except fluorene, phenanthrene, and fluoranthene. For these PAHs, the relationship was positive, which was ascribed to volatilization from various surfaces. The authors pointed out that the volume of traffic, which was the major source of NO, increased in summer months. NO participates in many atmospheric reactions that lead to formation of reactive atmospheric species able to degrade PAHs. Wania and McLachlan (2001) suggested that the reduction of atmospheric concentrations of PAHs in summer months could be caused by forest canopy uptake of SOC. If the canopy uptake was the only reason for the reduced air concentrations of organic air pollutants in summer, then the corresponding concentrations on leaves should be noticeably higher for summer months. However, Jouraeva et al. (2002) observed a similar double-peak trend for PAHs of a wide range of volatilities measured on linden leaves, with the lowest values of concentrations also corresponding to the warmest summer months (June and July). Interestingly,

this reduction was more pronounced for less volatile particulate PAHs with $\log K_{\text{OA}} > 10$.

Results of this research showed that PAH loads on linden and pear leaves followed two different trends (Fig. 4). These temperature-induced changes were more pronounced for HMW PAHs such as benzo(a)pyrene (Fig. 4, white solid area), their concentrations often followed an inverse temperature relationship which was not the case for predominantly fine-particle-accumulated lead (Fig. 2). Such trends for HMW PAHs are unlikely caused by changes in plant-air partitioning of these compounds (Komp and McLachlan, 1997). The reduction in leaf surface area owing to honeydew prevalence on linden leaves could have explained the decrease of the PAH concentrations in June, but not the temperature dependence over the entire growing season. One of the possible reasons for the reduced concentrations of MMW and HMW PAHs in summer is chemical and/or photochemical decomposition of particle-bound PAHs on leaves and in the air.

Linear regression analyses between daily mean concentrations of PAHs on linden leaves and various temperatures (sampling, night-time, and temperatures averaged over 1, 2, 3, 4, and 5 days prior to sampling) are given in Table 3. The burdens of some HMW PAHs on linden leaves were negatively correlated with the night-time temperatures (B(a)a: $p = 0.06$, $R^2 = 0.75$; B(b)f: $p = 0.08$, $R^2 = 0.70$; B(k)f: $p = 0.06$, $R^2 = 0.75$; B(a)p: $p = 0.05$, $R^2 = 0.76$; and Ind: $p = 0.09$, $R^2 = 0.68$) and to a lesser extent with the average temperatures of the sampling dates. The fact that this regression was prominent for particle-bound HMW PAHs but not for more volatile compounds is in agreement with Kotzias and Brussol's (1999) suggestion that PAHs adsorbed/absorbed onto particles' active surface undergo nitration more rapidly than those in the gaseous phase. Schauer et al. (2003) also came to the conclusion that 5- and 6-ring PAHs on airborne aerosol particles underwent substantial degradation that resulted in the formation of oxygenated and nitrated derivatives. Correlation of particle-bound PAHs with night-time but not day-time temperatures suggests that night-time decomposition of these carcinogenic compounds (US EPA, 1993)

Table 3

Linear regression analysis: mean concentrations of PAHs on linden tree leaves (over the growing season 2000) vs. the lowest temperatures (T) of the sampling day, sampling T , the average T of the sampling date, and T averaged over 2, 3, and 4 days prior to sampling at $\alpha = 0.10^a$

PAHs	P -values/% R^2 for T ($^{\circ}\text{C}$); $n = 5$					
	Lowest sampling	Sampling time	1-day average	2-day average	3-day average	4-day average
Flr	0.08/71^b	0.08/69	0.06/75	0.16/53	0.41/23	0.50/16
B(a)a	0.06/75	0.21/45	0.08/70	0.14/56	0.28/36	0.36/28
B(b)f	0.08/70	0.17/52	0.08/70	0.14/56	0.31/33	0.38/26
B(k)f	0.06/75	0.19/49	0.08/70	0.14/57	0.29/36	0.36/28
B(a)p	0.05/76	0.16/53	0.06/73	0.11/62	0.25/41	0.32/33
Ind	0.09/68	0.20/48	0.10/65	0.15/56	0.28/15	0.35/29

^a Results in bold indicate significant negative linear correlation at $\alpha = 0.10$.

^b % R^2 .

might be important. This idea is supported by the research results of Lewis et al. (2002), who studied formation and lifetime of particle-borne polycyclic aromatic compounds and discovered the night-time rise in nitro-PAHs. The authors attributed their observation to either reactions with ozone or the nitrate (NO_3) radical. Drastic reduction in particle-bound PAHs during summer months could also be explained by the fact that concentrations of the hydroxyl (OH) and NO_3 radicals in the atmosphere are dependent on temperature, ozone, and NO_2 concentrations, all of which are higher in summer months. The abundance of both, NO_3 and OH, radicals is strongly linked to ozone concentration, which increases during the warmest summer months with the increase in solar radiation.

Atkinson and Arey (1994) concluded that the dominant day-time loss of gas-phase PAHs was by reaction with the hydroxyl radical, but that both OH and NO_3 reactions (night-time degradation) contributed to the degradation of gas-phase PAHs. That could be the reason for linear regressions between more volatile, fluoranthene concentrations and both the sampling temperatures ($p = 0.08$) and the lowest temperatures of the sampling days ($p = 0.08$). Perhaps, the degradation of this compound is equally affected by the OH and NO_3 radical reactions, giving the strongest regression with the average T of the sampling day ($p = 0.06$).

It has been suggested that relative humidity plays an important role in degradation of PAHs. No direct correlation was observed between PAH concentrations and relative humidity in our experiment.

The results indicate the possibility of night-time decomposition of particle-bound PAHs accumulated on linden leaves. Set in the fluffy hyphae of sooty molds, PAHs could be susceptible to gaseous attacks from all directions by reactive atmospheric species like the nitrate radical. Negative correlations of the amounts of PAHs on linden leaves might also indicate possible degradation of particulate PAHs on the moldy leaf surface caused by utilization of these PAHs by sooty mold fungi as an available carbon source (Bennett and Faison, 1997; Doelle et al., 1992). In

addition, hyphae of these filamentous fungi are rich in lipids and solubility of PAHs in the hyphae lipids might be important in degradation of these often-carcinogenic compounds. It has been shown that sooty mold fungi can metabolize PAHs from a crystalline state due to the ability of tread-like hyphae to penetrate substrates that are unapproachable to bacteria or non-filamentous fungi (Bennett and Faison, 1997).

3.2.3. PAHs on *P. calleryana* leaves

Concentrations of most PAHs on pear tree leaves were slightly higher in early spring and in the fall. Unlike lead (Fig. 2, black solid area), B(a)p on pear leaves did not show a peak in July (Fig. 4, black solid area). Statistical analysis (Table 4) revealed a positive linear correlation between concentrations of some HMW PAHs and temperatures averaged over four days prior to sampling: B(b)f: $p = 0.08$, $R^2 = 0.70$; B(a)p: $p = 0.04$, $R^2 = 0.80$; B(g,h,i)p: $p = 0.07$, $R^2 = 0.72$. This result might mean that concentrations of these PAHs on pear leaves increased with the increase of temperature. Because these compounds have very high octanol-air partition coefficients, it is unlikely that their air concentration in the gaseous phase would change within four days to the extent that would impact the concentrations of these compounds on pear leaves. However, changes in temperature might result in changes of sorption behavior of a plant cuticle as proposed by Keymeulen et al. (1997), who studied plant-air partitioning of monocyclic organic compounds. The authors suggested that the enthalpy of phase change (air-plant cuticle) might not be a constant value over a range of temperatures. The change of the waxy cuticle sorption properties would probably be different for different plant species and would need to be further investigated.

The other important aspect that might have influenced the accumulation of HMW PAHs on pear leaves is the greater amount of epicuticular waxes on pear than on linden leaves (Jouraeva et al., 2002). PAH-containing fine particles might be deeply embedded in the waxy pear leaf surface, preventing PAHs from reacting with reactive atmospheric species. The explanation of these results is

Table 4

Linear regression analysis: concentrations of PAHs on pear tree leaves (over the growing season 2000) vs. the lowest temperature of the sampling day, the sampling T , the average T of the sampling day, and T averaged over 2, 3, 4, and 5 days prior to sampling at $\alpha = 0.10^a$

PAHs	P-values/% R^2 for T ($^{\circ}\text{C}$); $n = 5$						
	Lowest sampling	Sampling time	1-day average	2-day average	3-day average	4-day average	5-day average
B(b)f	0.45/18 ^b	0.48/18	0.46/19	0.31/36	0.12/64	0.08/70	0.21/45
B(a)p	0.31/33	0.31/33	0.34/40	0.24/36	0.06/75	0.04/80	0.13/59
B(ghi)p	0.50/17	0.51/16	0.52/15	0.33/64	0.12/64	0.07/72	0.26/39

^aResults in bold indicate significant positive linear regression at $\alpha = 0.10$.

^b% R^2 .

not trivial due to the existence of two counteracting processes: accumulation and degradation of particle-bound organic compounds on leaves.

4. Conclusions

The research results demonstrate that sooty mold fungi play an important role in accumulation of atmospheric air pollutants associated with fine particles. Leaves infested by sooty mold fungi trapped significantly higher amounts of both gaseous and particulate PAHs and heavy metals than leaves of the same species that were not affected by molds and can be instrumental in the removal of many toxins associated with atmospheric particulate matter.

Different regression trends (concentrations of PAHs vs. temperatures) for leaves of infested and non-infested species indicate that sooty mold fungi might alter the accumulation mode and/or degradation pathways of PAHs on leaves. Chemical decomposition of particle-bound PAHs, especially their night-time decay, needs to be further investigated. The possibility of enzymatic degradation of particle-bound PAHs while in close contact with sooty mold fungi is another important issue that needs to be studied.

From the human exposure point of view, high concentrations of PAHs and metals in the forest canopy can also diminish the potential of long-range transfer of these toxins by transporting harmful fine particles from the air to the soil. While in the soil, PAHs are less dangerous to human health, since plants cannot accumulate them via their root system (Sims and Overcash, 1983), and the products of their decomposition are chemically bound to soil and are not bioavailable (Kastner et al., 1999; Wang et al., 1990; Bollag et al., 1988; Bollag and Loll, 1983).

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